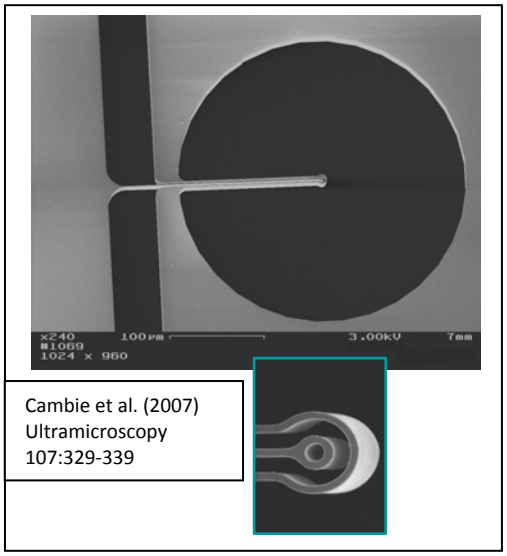
Life Sciences Division

E-Newsletter November 30, 2007

Highlights

NIH Grant Awarded for Development of Phase-Contrast Capability in Electron Microscopy

NIH awarded a new grant to LBNL on Oct. 1, 2007, the goal of which is to use modern microfabrication technology to create the equivalent of Zernike's quarter wave plate for microscopy, but in this case for electron microscopy rather than light microscopy. Micrometer-scale electrodes will be used to selectively apply a 90-degree phase shift to the unscattered electron wave at the back focal plane of the objective lens. Inspired by a suggestion first published in 1947, Jian Jin in the Bioinstrumentation Group at LBNL conceived of a practical design that is readily fabricated on the sub-micrometer scale. In this design the unscattered electrons are focused through the hole of a biased "drift tube", while a grounded electrode surrounding the biased electrode serves to shield the scattered electrons from the influence of the biased electrode. Tiny though this device is, it is still too large for use in a standard, commercially available electron microscope. To make this device useful for biological applications, therefore, a standard microscope will be modified with an additional relay lens that magnifies the diffraction pattern to a size that is commensurate with the scale of the microfabricated electrodes. At the same time, research will also be pursued to attempt the fabrication of the same design with features in the range of 50 nm, using facilities at the Molecular Foundry at LBNL. The principal driver for



Cambie et al. (2007) Ultramicroscopy 107:329-339

development of this technology is the DOE-funded GTL "Protein Complex Analysis Program (PCAP)" within the Life Sciences Division. It is projected that Zernike-type phase contrast will make it possible to use cryo-EM to obtain 3-D reconstructions of multiprotein complexes as small as 200 kDa or less, thereby greatly extending the coverage of the proteome to which this powerful technique can be applied. A second application will be to characterize the molecular changes that are produced in lignocellosic materials during attempts to "deconstruct" such materials and thereby render them more accessible to efficient, enzymatic hydrolysis. **Robert Glaeser**, an international leader in the field of electron cryo-microscopy, is the Principal Investigator of this project and a team member within PCAP.
Robert Glaeser, 11/07

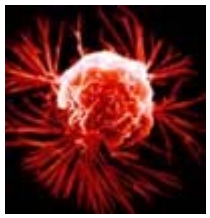
Cover Story: Discovery of Functional Elements in 12 Drosophila Genomes

Susan Celniker's and colleagues' article was featured as the cover story of Nature magazine's November 8, 2007 issue. "Sequencing of multiple related species followed by comparative genomics analysis constitutes a powerful approach for the systematic understanding of any genome. Here, we use the genomes of 12 Drosophila species for the de novo discovery of functional elements in the fly. Each type of functional element shows characteristic patterns of change, or 'evolutionary signatures', dictated by its precise selective constraints. Such signatures enable recognition of new protein-coding genes and exons, spurious and incorrect gene annotations, and numerous unusual gene structures, including abundant stop-codon readthrough. Similarly, we predict non-protein-coding RNA genes and structures, and new microRNA (miRNA) genes. We provide evidence of miRNA processing and functionality from both hairpin arms and both DNA strands. We identify several classes of pre- and post-transcriptional regulatory motifs, and predict individual motif instances with high confidence. We also study how discovery power scales with the divergence and number of species compared, and we provide general guidelines for comparative studies." Full story:
http://www.nature.com/nature/journal/v450/n7167/abs/nature06340.html;jsessionid=1594294486828E0819DF37030DD7EA98
Susan Celniker, 11/07

Cover Story: A Critical Cog in the DNA Repair Machinery: Understanding RAD51AP1

A major publication from **Claudia Wiese** and **David Schild** in Molecular Cell was selected as the cover story for the November 9, 2007 issue. The paper describes and extensively characterizes a novel player in homologous recombinational repair, the human protein RAD51AP1 (Associated Protein 1), and shows it to be important for repair of ionizing radiation-induced DNA damage and of DNA interstrand crosslinks, as well as for maintaining genome stability both with and without exogenous damage. Biochemical studies reported in the paper reveal RAD51AP1 to be one of only a few proteins that function in homologous recombination downstream of the formation of the RAD51 filament.
Full story: http://www.lbl.gov/Science-Articles/Archive/sabl/2007/Nov/RAD51AP1.html
Priscilla Cooper, 11/07

Multi-Level Targeting in Breast Cancer Cells



Breast cancer cell

The chemical signaling pathways that control the life cycle of cells offer many important targets for researchers hoping to stop tumor growth. Yet the complex nature of these pathways may make it impossible to kill a cell with a single therapeutic bullet, as researchers from UC San Francisco, **Berkeley Lab (Joe Gray et. al),** and other institutions discovered in a study of breast cancer cells. Their findings suggest that molecules used to inhibit the MEK protein can "switch on" another pathway that keeps cancer cells from dying. Their solution is to look elsewhere to see where these pathways intersect. They have uncovered two targets that, when chemically inhibited at the same time, caused apoptosis.

Full story: http://www.sciencedaily.com/releases/2007/10/071024115312.htm

Today at Berkeley Lab, 10/30/07

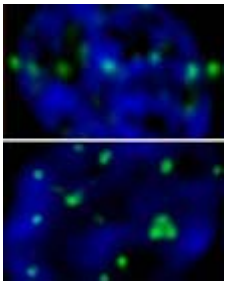
Russian Steam Spurts Again after Landslide

Valley of Geysers

Four months after a landslide dumped 4.5 million cubic meters of rock and mud into Russia's Valley of Geysers, two geysers have re-emerged, and others may be on their way, researchers say. Initial reports of the landslide worried **Tamas Torok**, a microbiologist at Berkeley Lab who regularly visits the region to study its unique microbial climate. “They said the Valley of Geysers was destroyed,” he says. But on a recent visit, he found that many of his research sites had escaped unharmed. Full story:
http://www.nature.com/news/2007/071003/full/449519a.html

Today at Berkeley Lab, 11/6/2007

Broken DNA Moved to ‘Repair Shops’



Mapped DNA repair sites

When high-speed particles of space radiation zip through an astronaut's body, they will occasionally strike and break a strand of DNA. Scientists have long thought that enzymes always go to the site of injury and fix the DNA damage in place, but new research suggests that cells might sometimes move broken DNA to special "repair shops" instead. As part of this research, a team led by Berkeley Lab life scientist **Sylvain Costes** simulated space radiation by exposing lab-grown human cells to one of three radiation types: gamma rays, X-rays, and high-energy iron nuclei generated in the particle accelerator at NASA's Space Radiation Laboratory. Full story:
http://www.yubanet.com/artman/publish/article_69867.shtml

Today at Berkeley Lab, 11/9/07

Manager Development Class Completes Year

Twenty participants in the Berkeley Lab Institute's Management Development Program completed a year's worth of training and were recently honored in Perseverance Hall for their accomplishments. The leaders from scientific, engineering and operations programs devoted one day a month to developing their skills in various aspects of management.

Front row, left to right: Ross Schlueter, John Chernowski, Richard Takahashi, Rachel Carl; second row: Tony Warwick, Nance Matson, **Mary Helen Barcellos-Hoff**, BLI manager Karen Ramorino; third row: Len Pennacchio, John Dahlgard, David Hathaway, Richard DeBusk, Daniela Leitner, Richard Gregory. Missing when the photo was taken: Musa Ahmed, Jonathan Carter, David Chen, Brent Draney, Michael Martin, GianLuca Sabbi, and Viviana Wolinsk

Today at Berkeley Lab, 11/20/07

Jack-o-Lantern Honors Berkeley Lab's 76 Years

Life scientist **Henry Marr**, a member of Division Director **Joe Gray**'s lab, stayed up until the wee hours of the morning yesterday carving this Halloween homage to the Lab's 76 years of existence.

Today at Berkeley Lab, 11/1/07

Age Research: A New Angle on Getting 'Old'

The Buck Institute in Novato, CA was founded on the premise that aging and disease are manifestations of the same biological processes, and they can be understood only by working across disciplines. In the 1950s, aging was thought to be intractable and there weren't a lot of ways to study it beyond simple observation. "People were essentially grinding up old and young rats" and coming up with descriptions rather than learning about mechanics, says **Judith Campisi**, a cellular and molecular biologist who splits her time between the Buck and the Life Sciences Division at Berkeley Lab.

Full story: http://www.nature.com/news/2007/071128/full/450603a.html

Today at Berkeley Lab, 11/30/07

Maternal Repair of DNA Damage in the Fertilizing Sperm

Francesco Marchetti and colleagues have demonstrated that disrupting maternal repair in the egg results in a significant increase in the frequencies of zygotes with sperm-derived chromosomal aberrations following paternal exposure to ionizing radiation (Marchetti et al 2007 PNAS 104: 17725-11129, November 7). This research in mice also demonstrates that the risks of pregnancy loss and birth of offspring with chromosomal defects after paternal exposure to ionizing radiation depend on the

ability of the maternal genome to repair DNA damage carried in the fertilizing sperm. Both Lawrence Livermore National Laboratory (where the experiments were conducted) and LBNL (where the analyses were conducted) have highlighted the research in their newsletters. (contact: Francesco Marchetti, fmarchetti@lbl.gov)
Andy Wyrobek, 11/07

Gene Expression Bioinformatics Workshop
Sanchita Bhattacharya, organized and chaired the bioinformatics workshop entitled "Advances in Bioinformatics Methods for the Analyses of Global Gene Expression Networks and Pathways" that was held during the recent annual meeting of the Environmental Mutagen Society in Atlanta Georgia (October 22, 2007). This workshop highlighted the interface between genomic technologies and new bioinformatics tools that can now enable advances in cellular mechanism science and molecular target discovery. It presented new statistical and bioinformatics approaches for the analyses of gene expression data to identify the underlying cellular networks and biochemical pathways. There were about 65 attendees. (contact: Sanchita Bhattacharya, SBhattacharya@lbl.gov*)*
Andy Wyrobek, 11/07

Director of the NASA Space Radiation Summer School for 2008
Eleanor Blakely, is the new Director of the NASA Space Radiation Summer School to be held at Brookhaven National Laboratory in June 2008. This long-standing course provides an integrated curriculum of radiation biology, radiation chemistry, and physics culminating in hands-on accelerator-based experiments. Course topics range from early molecular response mechanisms to carcinogenesis and late degenerative effects following exposure to space radiation. The course also covers topics of the space radiation environment, physics and biochemistry of charged particle interaction with condensed matter, ionizing radiation dosimetry, and accelerator operations. Course faculty will consist of leading university and national laboratory biologists and physicists actively engaged in NASA space radiation research and BNL experts in heavy ion experimentation and methods. (contact: Eleanor Blakely, EABlakely@lbl.gov)
Andy Wyrobek, 11/07

Environmental Mutagen Society
Andrew J. Wyrobek was Scientific Program Chair of the annual meeting of the Environmental Mutagen Society, held in Atlanta Georgia (October 20-24). The theme was "Mutational and Epigenetic Mechanisms of Susceptibility and Risks for Genetic Diseases", and the meeting attracted about 500 attendees from 20 countries, representing scientists from industry, government and academia. The Environmental Mutagen Society (EMS) is the primary scientific society fostering research on the basic mechanisms of mutagenesis and epigenetics and the application of this knowledge to the field of genetic toxicology and risk assessments for genetic diseases including cancer. Andrew J. Wyrobek is serving a one-year term as EMS president, and leading the 10-year strategic planning exercise for the society. **Priscilla Cooper** is the new president-elect and program chair for EMS 2008 to be held in Puerto Rico in October 2008. (contact: Andrew J. Wyrobek, ajwyrobek@lbl.gov)
Andy Wyrobek, 11/07

Recent publications (selected)

Barcellos-Hoff MH. Cancer as an emergent phenomenon in systems radiation biology, Radiat Environ Biophys. 2007 Nov 20; PMID: 18026977

Radiation-induced DNA damage elicits dramatic cell signaling transitions, some of which are directed towards deciding the fate of that particular cell, while others lead to signaling to other cells. Each irradiated cell type and tissue has a characteristic pattern of radiation-induced gene expression, distinct from that of the unirradiated tissue and different from that of other irradiated tissues. It is the sum of such events, highly modulated by genotype that sometimes leads to cancer. The challenge is to determine as to which of these phenomena have persistent effect that should be incorporated into models of how radiation increases the risk of developing cancer. The application of systems biology to radiation effects may help to identify which biological responses are significant players in radiation carcinogenesis. In contrast to the radiation biology paradigm that focuses on genomic changes, systems biology seeks to integrate responses at multiple scales (e.g. molecular, cellular, organ, and organism). A key property of a system is that some phenomenon emerges as a property of the system rather than of the parts. Here, the idea that cancer in an organism can be considered as an emergent phenomenon of a perturbed system is discussed. Given the current research goal to determine the consequences of high and low radiation exposures, broadening the scope of radiation studies to include systems biology concepts should benefit risk modeling of radiation carcinogenesis.

Pluth JM, Yamazaki V, Cooper BA, Rydberg BE, Kirchgessner CU, **Cooper PK**. DNA double-strand break and chromosomal rejoining defects with misrejoining in Nijmegen breakage syndrome cells. DNA Repair (Amst). 2007 Oct 3PMID: 17919995

NBS1-deficient cells exhibit pronounced radiosensitivity and defects in chromosome integrity after ionizing radiation (IR) exposure, yet show only a minor defect in DNA double-strand break (DSB) rejoining, leaving an as yet unresolved enigma as to the nature of the radiosensitivity of these cells. To further investigate the relationship between radiosensitivity, DSB repair, and chromosome stability, we have compared cytological and molecular assays of DSB misrejoining and repair in NBS1-defective, wild type, and NBS1-complemented cells after IR damage. Our findings suggest a subtle defect in overall DSB rejoining in NBS1-defective cells and uniquely also reveal reduced ability of NBS1-defective cells to rejoin correct ends of DSBs. In agreement with published results, one of two different NBS1-defective cell lines showed a slight defect in overall rejoining of DSBs compared to its complemented counterpart, whereas another NBS line did not show any difference from wild type cells. Significant defects in the correct rejoining of DSBs compared to their respective controls were observed for both NBS1-defective lines. The defect in DSB rejoining and the increased misrejoining detected at the molecular level were also reflected in higher levels of fragments and translocations, respectively, at the chromosomal level. This work provides both molecular and cytological evidence that NBS1-deficient cells have defects in DSB processing and reveals that these molecular events can be manifest cytologically.

Comolli LR, Spakowitz AJ, Siegerist CE, Jardine PJ, Grimes S, Anderson DL, Bustamante C, **Downing KH**. Three-dimensional architecture of the bacteriophage phi29 packaged genome and elucidation of its packaging process. Virology. 2007 Nov 12; PMID: 18001811

The goal of the work reported here is to understand the precise molecular mechanism of the process of DNA packaging in dsDNA bacteriophages. Cryo-EM was used to directly visualize the architecture of the DNA inside the capsid and thus to measure fundamental physical parameters such as inter-strand distances, local curvatures, and the degree of order. We obtained cryo-EM images of bacteriophage that had packaged defined fragments of the genome as well as particles that had partially completed the packaging process. The resulting comparison of structures observed at intermediate and final stages shows that there is no unique, deterministic DNA packaging pathway. Monte Carlo simulations of the packaging process provide insights on the forces involved and the resultant structures

Rohmer D, Sitek A, **Gullberg GT.** Reconstruction and visualization of fiber and laminar structure in the normal human heart from ex vivo diffusion tensor magnetic resonance imaging (DTMRI) data. Invest Radiol. 2007 Nov;42(11):777-89. PMID: 18030201

The human heart is composed of a helical network of muscle fibers organized to form sheets that are separated by cleavage planes responsible for the orthotropic mechanical properties of cardiac muscle. The purpose of this study is the reconstruction and visualization of these structures in 3 dimensions. METHODS: Anisotropic least square filtering followed by fiber and sheet tracking techniques were applied to diffusion tensor magnetic resonance imaging data of the excised human heart. Fibers were reconstructed using the first eigenvectors of the diffusion tensors. The sheets were reconstructed using the second and third eigenvectors and visualized as surfaces. RESULTS: The fibers are shown to lie in sheets that have transmural structure, which correspond to histologic studies published in the literature. Quantitative measurements show that the sheets as appose to the fibers are organized into laminar orientations without dominant populations. CONCLUSIONS: A visualization algorithm was developed to demonstrate the complex 3-dimensional orientation of the fibers and sheets in human myocardium.

Williams PT, Franklin B. Vigorous Exercise and Diabetic, Hypertensive, and Hypercholesterolemia Medication Use. Med Sci Sports Exerc. 2007 Nov;39(11):1933-1941. PMID: 17986900

The prevalences of diabetes, hypertension, and high cholesterol all decrease with increased levels of physical activity and cardiorespiratory fitness. Whether these reductions extend beyond contemporary guideline activity levels and whether fitness affects medication use independent of activity, remains unclear. METHODS:: Cross-sectional analyses of 62,291 male and 45,041 female runners, of whom 496 used antidiabetic, 3738 used antihypertension, and 2360 used low-density lipoprotein cholesterol (LDL-C)-lowering medications. Cardiorespiratory fitness was reported as speed (m.s) during a 10-km foot race. RESULTS:: Medication use was significantly inversely associated with activity and fitness ($P < 0.001$, except LDL-C-lowering versus women's fitness). Compared with </= 16 km.wk (guideline levels), the odds in men and women who ran > 64 km.wk were, respectively, 69% and 55% lower for antidiabetic, 48% and 52% lower for antihypertension, and 64% and 51% lower for LDL-C-lowering medication use. Compared with the least-fit men (< 3.25 m.s) and women (< 2.8 m.s), the odds for those who were most fit (men > 4.75 m.s; women > 4.0 m.s) were 58% and 65% lower for antidiabetic, and 76% and 55% lower for antihypertensive medication use. Odds for LDL-C-lowering medication use were 87% lower in the fittest versus the least-fit men. Adjustment for activity only

moderately diminished the inverse relationships of fitness with medication use. CONCLUSION:: Among individuals who exceed current guideline levels, antidiabetic, antihypertension, and LDL-C-lowering medications are inversely related to vigorous physical activity and cardiorespiratory fitness. Lower odds of medication use with higher fitness occur independently of physical activity.

Huang B, Brennan KM, **Budinger TF**, Maltz JS. Assessment of Andothelial Function in the Radial Artery Using Inhaled Albuterol. Conf Proc IEEE Eng Med Biol Soc. 2007;1:3629-31. PMID: 18002782

Endothelial dysfunction is an early indicator of developing atherosclerosis and is a strong predictor of future heart attack and stroke. At present, evaluation of endothelial function (EF) (specifically, EF mediated by nitric oxide, NO) is too technically difficult to form part of a routine clinical examination. Non-invasive methods that measure NO-dependent EF in arteries make use of a 4-5 minute blood pressure cuff occlusion of the arm in order to induce reactive hyperemia (RH) upon cuff release. The increased blood flow that results from the RH stimulates the endothelial cells to release NO and relax the surrounding vascular smooth muscle. The magnitude of the change in arterial caliber or stiffness provides a measure of EF. The cuff occlusion is uncomfortable and inflation and release inevitably move the arm, increasing the technical difficulty of obtaining reliable measurements. In Beta<inf>2</inf>-adrengergic agonist albuterol induces NO-mediated vasorelaxation in resistance vessels of humans. We examine, for the first time, the effect of albuterol on conduit vessels (radial artery) by measuring changes in the transit times of artificial pulses observed after inhalation of albuterol. We conclude that albuterol is able to relax the radial artery and that this correlates with the effects of RH (r=0.62, p=0.04). However, the response to a dose of 360 mu-g is smaller and more variable when compared to the response to RH-based stimulus.

Peng Q, **Budinger TF**. ZigBee-based Wireless Intra-oral Control System for Quadriplegic Patients. Conf Proc IEEE Eng Med Biol Soc. 2007;1:1647-50. PMID: 18002289

A human-to-computer system that includes a wireless intra-oral module, a wireless coordinator and distributed wireless controllers, is presented. The state-of-the-art ZigBee protocol is employed to achieve reliable, low-power and cost-efficient wireless communication between the tongue, computer and controllers. By manipulating five buttons on the wireless intra-oral module using the tongue, the subject can control cursors, computer menus, wheelchair, lights, TV, phone and robotic devices. The system is designed to improve the life quality of patients with stroke and patients with spinal cord injury.

Nikanjam M, Gibbs AR, Hunt CA, **Budinger TF**, Forte TM. Synthetic nano-LDL with paclitaxel oleate as a targeted drug delivery vehicle for glioblastoma multiforme. J Control Release. 2007 Dec 20;124(3):163-71. Epub 2007 Sep 26. PMID: 17964677

The low density lipoprotein (LDL) receptor has been shown to be upregulated in GBM tumor cells in vitro and is therefore a potential molecular target for the delivery of therapeutic agents. A synthetic nano-LDL (nLDL) particle was developed as a drug delivery vehicle targeted to GBM cells by incorporating a lipophilic prodrug, paclitaxel oleate, into the particle. Nano-LDL containing paclitaxel oleate (nLDL-PO) was constructed by combining a synthetic peptide

containing a lipid binding motif and the LDL receptor (LDLR) binding domain of apolipoprotein B-100 with a lipid emulsion consisting of phosphatidyl choline, triolein, and paclitaxel oleate. Paclitaxel oleate incorporated into the core of the lipid particle. nLDL-PO cell survival in GBM cell lines was found to be time, concentration, and cell line dependent. Cell killing was observed with short drug incubations and exhibited saturation at 6 h. nLDL-PO cell survival improved in the presence of the LDL receptor inhibitor, suramin, demonstrating that the drug was delivered via the LDL receptor. Collectively, these data strongly suggest that the synthetic nano-LDLs can incorporate lipophilic drugs and are capable of killing GBM cells. nLDL-PO has the potential to serve as a selective drug delivery vehicle for targeting GBM tumors via the LDL receptor.

Park MJ, **Downing KH**, Jackson A, Gomez ED, Minor AM, Cookson D, Weber AZ, Balsara NP. Increased Water Retention in Polymer Electrolyte Membranes at Elevated Temperatures Assisted by Capillary Condensation**.** Nano Lett. 2007 Nov 14;7(11):3547-3552 PMID: 17960948

We establish a new systematic methodology for controlling the water retention of polymer electrolyte membranes. Block copolymer membranes comprising hydrophilic phases with widths ranging from 2 to 5 nm become wetter as the temperature of the surrounding air is increased at constant relative humidity. The widths of the moist hydrophilic phases were measured by cryogenic electron microscopy experiments performed on humid membranes. Simple calculations suggest that capillary condensation is important at these length scales. The correlation between moisture content and proton conductivity of the membranes is demonstrated.

Wille H, Govaerts C, Borovinskiy A, Latawiec D, Downing KH, Cohen FE, Prusiner SB. Electron crystallography of the scrapie prion protein complexed with heavy metals. Arch Biochem Biophys. 2007 Nov 15;467(2):239-48. PMID: 17935686

The insolubility of the disease-causing isoform of the prion protein (PrP(Sc)) has prevented studies of its three-dimensional structure at atomic resolution. Electron crystallography of two-dimensional crystals of N-terminally truncated PrP(Sc) (PrP 27-30) and a miniprion (PrP(Sc)106) provided the first insights at intermediate resolution on the molecular architecture of the prion. Here, we report on the structure of PrP 27-30 and PrP(Sc)106 negatively stained with heavy metals. The interactions of the heavy metals with the crystal lattice were governed by tertiary and quaternary structural elements of the protein as well as the charge and size of the heavy metal salts. Staining with molybdate anions revealed three prominent densities near the center of the trimer that forms the unit cell, coinciding with the location of the beta-helix that was proposed for the structure of PrP(Sc). Differential staining also confirmed the location of the internal deletion of PrP(Sc)106 at or near these densities

Killilea AN, **Downing KH**, Killilea DW. Zinc deficiency reduces paclitaxel efficacy in LNCaP prostate cancer cells. Cancer Lett. 2007 Dec 8;258(1):70-9. PMID: 17905512

Chemotherapeutics used to treat prostate cancer are often from a class of drugs that target microtubule networks, such as paclitaxel. A previous report indicated that supplemental zinc sensitized prostate cancer cells to paclitaxel-induced apoptosis, suggesting that increased zinc levels might enhance paclitaxel efficacy. The effect of zinc deficiency on paclitaxel activity is not known though, so we tested this in two prostate cancer cell lines maintained under moderately

zinc-deficient conditions. LNCaP and PC3 cell lines were used as models of early and late-stage prostate cancer, respectively. Cells cultured in reduced zinc levels did not demonstrate altered cell viability, growth rates, or intracellular zinc content. Additionally, zinc deficiency alone had no apparent effect on cell cycle kinetics or apoptosis levels. However, the IC(50) for paclitaxel-induced cell cycle arrest increased in LNCaP cells from zinc-deficient compared to zinc-replete conditions. Consequently, paclitaxel-induced apoptosis was reduced in LNCaP cells from zinc-deficient compared to zinc-replete conditions. In PC3 cells, the effects of paclitaxel were independent of zinc status. Reduced extracellular zinc levels were shown to affect paclitaxel activity in a prostate cancer cell line. Given the prevalence of zinc deficiency, determining how chemotherapeutic action is modulated by zinc adequacy may have clinical importance.

Amat F, Moussavi F, Comolli LR, Elidan G, **Downing KH,** Horowitz M. Markov random field based automatic image alignment for electron tomography. J Struct Biol. 2007 Jul 28; PMID: 17855124

We present a method for automatic full-precision alignment of the images in a tomographic tilt series. Full-precision automatic alignment of cryo electron microscopy images has remained a difficult challenge to date, due to the limited electron dose and low image contrast. These facts lead to poor signal to noise ratio (SNR) in the images, which causes automatic feature trackers to generate errors, even with high contrast gold particles as fiducial features. To enable fully automatic alignment for full-precision reconstructions, we frame the problem probabilistically as finding the most likely particle tracks given a set of noisy images, using contextual information to make the solution more robust to the noise in each image. To solve this maximum likelihood problem, we use Markov Random Fields (MRF) to establish the correspondence of features in alignment and robust optimization for projection model estimation. The resulting algorithm, called Robust Alignment and Projection Estimation for Tomographic Reconstruction, or RAPTOR, has not needed any manual intervention for the difficult datasets we have tried, and has provided sub-pixel alignment that is as good as the manual approach by an expert user. We are able to automatically map complete and partial marker trajectories and thus obtain highly accurate image alignment. Our method has been applied to challenging cryo electron tomographic datasets with low SNR from intact bacterial cells, as well as several plastic section and X-ray datasets.

Lin MF, Carlson JW, Crosby MA, Matthews BB, Yu C, Park S, Wan KH, Schroeder AJ, Gramates LS, St Pierre SE, Roark M, Wiley KL Jr, Kulathinal RJ, Zhang P, Myrick KV, Antone JV, **Celniker SE**, Gelbart WM, Kellis M. Revisiting the protein-coding gene catalog of Drosophila melanogaster using 12 fly genomes. Genome Res. 2007 Nov 7; PMID: 17989253

The availability of sequenced genomes from 12 Drosophila species has enabled the use of comparative genomics for the systematic discovery of functional elements conserved within this genus. We have developed quantitative metrics for the evolutionary signatures specific to protein-coding regions and applied them genome-wide, resulting in 1193 candidate new protein-coding exons in the D. melanogaster genome. We have reviewed these predictions by manual curation and validated a subset by directed cDNA screening and sequencing, revealing both new genes and new alternative splice forms of known genes. We also used these evolutionary signatures to evaluate existing gene annotations, resulting in the validation of 87% of genes lacking descriptive names and identifying 414 poorly conserved genes that are likely to be spurious predictions, noncoding, or species-specific genes. Furthermore, our methods

suggest a variety of refinements to hundreds of existing gene models, such as modifications to translation start codons and exon splice boundaries. Finally, we performed directed genome-wide searches for unusual protein-coding structures, discovering 149 possible examples of stop codon readthrough, 125 new candidate ORFs of polycistronic mRNAs, and several candidate translational frameshifts. These results affect >10% of annotated fly genes and demonstrate the power of comparative genomics to enhance our understanding of genome organization, even in a model organism as intensively studied as Drosophila melanogaster.